

## REVIEW

# Structure and functions of arrestins

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## Abstract

Transmembrane signal transductions in a variety of cell types that mediate signals as diverse as those carried by neurotransmitters, hormones, and sensory signals share basic biochemical mechanisms that include: (1) an extracellular perturbation (neurotransmitter, hormone, odor, light); (2) specific receptors; (3) coupling proteins, such as G proteins; and (4) effector enzymes or ion channels. Parallel to these amplification reactions, receptors are precisely inactivated by mechanisms that involve protein kinases and regulatory proteins called arrestins. The structure and functions of arrestins are the focus of this review.

**Keywords:** arrestins; protein kinases; receptor inactivation; transmembrane signal transduction

Over 100 G protein-coupled receptors, when stimulated, are known to modulate concentrations of second-messengers, changes in ion-channel conductance, or other cellular events. The amplification of a stimulus-mediated signal is achieved (1) at the level of the agonist-receptor activation of several hundred G proteins, and (2) at the level of the enzymatic/conductance activity of the effector molecules that are modulated by the G protein. Deactivation of the receptor involves G protein-coupled receptor kinases (GRKs) (reviewed by Inglese et al., 1993), other protein kinases, and regulatory proteins called arrestins. The binding of arrestin to phosphorylated agonist-occupied receptor (e.g., photolyzed phosphorylated rhodopsin) quenches activation of the G protein ( $G_T$ , transducin). This review describes the biochemical, physiological, and structural properties of the arrestin family and discusses our current understanding of the function of these molecules.

Arrestin causes an autoimmune disease, uveoretinitis, that resembles uveitis in humans (Wacker et al., 1977). Immunization of experimental animals with arrestin, also known as S-antigen, results in inflammation of the uvea and retina, followed by destruction of retinal photoreceptor cells (reviewed in Shinohara et al., 1988). Arrestin has also been implicated in multiple sclerosis (Ohguro et al., 1993a). These aspects of arrestin are beyond the scope of this review.

## Sequence information

Initial collaborative efforts between the laboratories of Drs. L. Donoso and T. Shinohara resulted in the cloning of retinal bovine arrestin. The deduced amino acid sequence of this monomeric protein contains 404 amino acids (45,275 Da) and encompasses peptide sequences derived from purified arrestin (Shinohara et al., 1987a). Soon after, the sequence of retinal arrestin from many species was determined (Shinohara et al., 1992), and more recently, a retinal cone-specific homolog has been cloned (Murakami et al., 1993; Craft et al., 1994).

The most important information gained from these studies is that, although overall sequence similarity between arrestins from different species is ~90%, the degree of similarity drops to ~60% and ~45% at the N- and C-termini, respectively. The C-terminal region of arrestin are not involved directly in the interaction with the receptor, but may play a regulatory role in the structure and function of arrestin. This conclusion is based on the fact that the rhodopsin sequence differs only slightly between species.

The gene for *Drosophila* photoreceptor-specific arrestin encodes a 364-amino acid protein, termed arrestin I, that shares ~40% amino acid sequence identity with human and bovine arrestin and lacks the C-terminal region (Fig. 1) (Hyde et al., 1990; Smith et al., 1990). A second eye-specific gene encoding a 401-amino acid protein, termed arrestin II, that exhibits ~42% amino acid sequence identity with bovine arrestin was recently identified (LeVine et al., 1990; Yamada et al., 1990) (Fig. 1).

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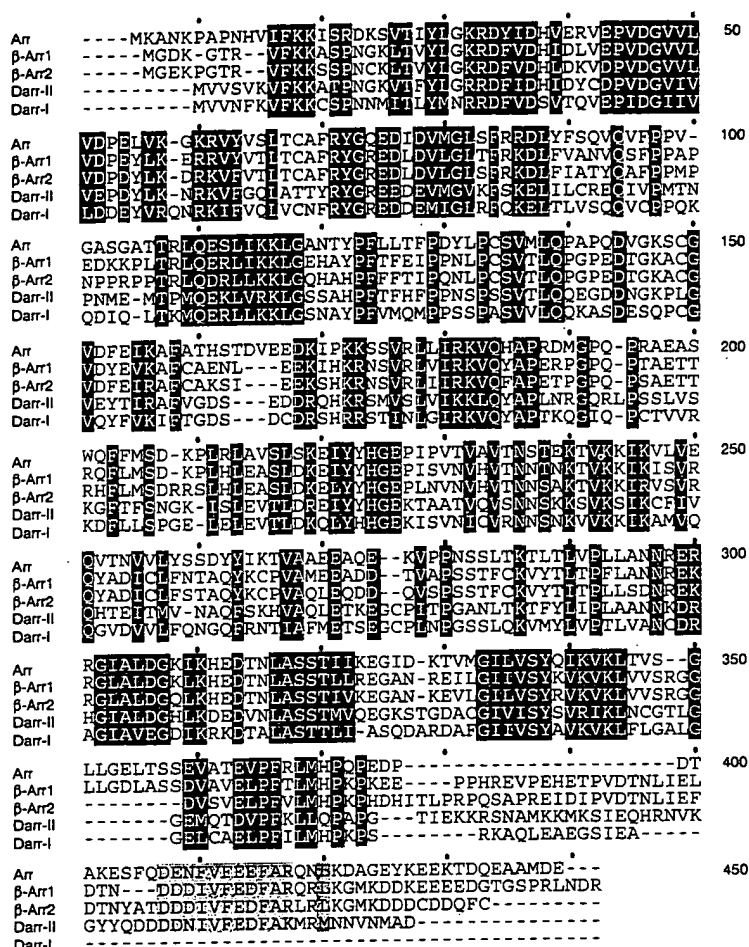


Fig. 1. Sequence alignment of bovine arrestin with its homologs. The bovine arrestin sequence (Arr; Shinohara et al., 1987a) was aligned with bovine  $\beta$ -arrestin-1 ( $\beta$ -Arr1; Lohse et al., 1990), rat  $\beta$ -arrestin-2 ( $\beta$ -Arr2; Attramadal et al., 1992), and *Drosophila melanogaster* arrestin I (Darr-I; Smith et al., 1990) and II (Darr-II; Hyde et al., 1990). Identical or highly homologous residues are indicated by white letters, the sequence homology at the C-terminal region between longer forms of arrestins are indicated by shading. The sequence alignment shows that arrestins are phylogenetically conserved (bovine and *Drosophila*) and that arrestins from different transduction systems share strong sequence homology (retinal arrestin and  $\beta$ -arrestins). The sequence homology is present throughout the sequence with the exceptions of the N- and C-termini.

For some time it was known that phosphorylation of the  $\beta_2$ -adrenergic receptor was not sufficient for rapid receptor desensitization and that a novel protein must be involved to augment the quenching signal initiated by receptor kinases. By analogy to phototransduction, an arrestin-like protein appears to be an ideal candidate. The cDNA for such a cofactor has been cloned and found to encode a 418-amino acid protein homologous to the retinal protein arrestin, termed  $\beta$ -arrestin-1 (Lohse et al., 1990). Furthermore, utilizing a low-stringency hybridization technique, Lefkowitz's group isolated a second cDNA clone representing a distinct  $\beta$ -arrestin-like gene, termed  $\beta$ -arrestin-2 (Attramadal et al., 1992), which exhibits ~80% amino acid sequence identity with  $\beta$ -arrestin-1 (Fig. 1).  $\beta$ -Arrestin-1 and  $\beta$ -arrestin-2 are ubiquitous proteins, yet predominantly localized in neuronal tissues and in the spleen. In the central nervous system,  $\beta$ -arrestin-2 appears to be more abundant than  $\beta$ -arrestin-1, whereas the neuronal distribution of  $\beta$ -arrestin-1 and  $\beta$ -arrestin-2 shows extensive and heterogenous labeling of the 2 proteins. Human and bovine homologs of  $\beta$ -arrestin-1 and  $\beta$ -arrestin-2 were recently cloned (Rapoport et al., 1992; Parruti et al., 1993; Sterne-Marr et al., 1993).

There is no overall sequence similarity between arrestins and other proteins. However, it has been proposed that arrestins contain several regions of sequence homology with the  $\alpha$ -subunit of G proteins, including a putative receptor binding site (Shinohara et al., 1987a; Lohse et al., 1990). Although it is an intriguing

possibility that the interaction between the activated receptor and G protein (heterotrimeric GTP-binding protein) or arrestin (monomeric protein) utilizes the same structural motif, the proposed sequence homology is located on small stretches of sequences that are in reverse order on both proteins, and is statistically insignificant.<sup>1</sup> Thus, it is not known whether this sequence homology is relevant for arrestin function. However, Komori et al. (1988) have found that arrestin is ADP-ribosylated by cholera toxin, similar to the  $\alpha$ -subunit of  $G_T$ . The authors speculated that because arrestin exhibits sequence homology with the ADP-ribosylation sites of  $G_{T\alpha}$ , it must also resemble  $G_{T\alpha}$  in its tertiary structure in a larger cholera toxin recognition domain. In addition, striking sequence homology is observed between regions 70–83 and 361–374 of the arrestin sequence and the  $Ca^{2+}$ -binding loops of calmodulin and troponin C. However, the  $\alpha$ -helices necessary for  $Ca^{2+}$  binding that flank these loops in calmodulin and troponin C are not present in the arrestin sequence, perhaps explaining why arrestin does not bind  $Ca^{2+}$  (Palczewski & Hargrave, 1991).

<sup>1</sup> For example,  $\beta$ -arrestin sequences <sup>74</sup>TFRKDL, <sup>94</sup>KK-PLT, <sup>291</sup>GKLLKHE, <sup>379</sup>LDTNDDDIVF, and <sup>402</sup>DKEEEDG were postulated to be homologous to the  $G_{\alpha}$  sequences (given in the same order): <sup>191</sup>TF-KNL, <sup>280</sup>KKSPLT, <sup>280</sup>KKSPLTI, <sup>327</sup>TDNNIQVVF, and <sup>20</sup>EKNLKEDG.

## Gene organization

The human and mouse arrestin genes are ~50 kilobase pairs (kbp) in length and are comprised of 16 exons (10–100 bp, 3% of the gene) and 15 introns (>2 kbp each, 97% of the gene) (Tsuda et al., 1988, 1991; Yamaki et al., 1990). Human arrestin (403 amino acid residues) was translated from a mRNA of 1.9 kbp. The 5'-flanking region of the gene, ~1.1 kbp, has no known regulatory elements for transcription but displayed promoter activity in an in vitro transcription assay. Promoter sequence positions -38 to +304 are sufficient to direct low levels of retina-specific gene expression, whereas sequences extending upstream to position -209 support higher levels of expression in the retina and detectable expression in the lens, pineal gland, and brain (Breitman et al., 1991).

The arrestin gene is localized on chromosome 2 in humans, and chromosome 1 in mouse (Danciger et al., 1989; Ngo et al., 1990). These results suggest that the arrestin gene does not localize to sites of known mutations related to retinal degenerative diseases. However, the gene for human cone arrestin maps to the proximal long arm of the X chromosome, making it a candidate for several inherited X-linked retinopathies (Craft et al., 1994).

## Splice variants

Two alternatively spliced isoforms of human  $\beta$ -arrestin-1 and arrestin were recently identified. These new variants differ only in the absence of 24 bp/8 amino acids within the C-terminal region (Parruti et al., 1993). Both isoforms have been found in all tissues tested. A  $\beta$ -arrestin-2 splice variant contains an 11-amino acid insert between residues 361 and 362 (Sterne-Marr et al., 1993). The functional differences between these forms of arrestins await further investigation.

We recently isolated another splice variant of arrestin, termed  $p^{44}$ , that is truncated at the C-terminus (Palczewski et al., 1994; Smith et al., 1994).  $p^{44}$  is specifically localized in the photoreceptor outer segments, the site of phototransduction, whereas arrestin is also present in other retinal cells. It is conceivable that as many as 8 arrestin variants may be present in some tissues (such as retina).

## Posttranslational modifications

It has been reported that bovine arrestin may contain lipids and carbohydrate moieties covalently attached to the protein (for a review, see Shinohara et al., 1988). Mass spectroscopic analysis reveals that arrestin does not have posttranslational modifications other than N-terminal acetylation (H. Ohguro, K. Palczewski, K.A. Walsh, & R. Johnson, in prep.). All 3 arrestin cysteine residues are available as free sulfhydryl groups (Pogozheva et al., 1989; Palczewski et al., 1992a) rather than in disulfide linkage, as previously suggested (Shinohara et al., 1987a). Retinal arrestin consists of several isoelectric subspecies ( $pK_a$  5.5–6.2) (Weyand & Kühn, 1990). In the presence of rod outer segment membranes, arrestin is phosphorylated by protein kinase C (0.2 mol phosphate/mol arrestin), perhaps accounting for some of the heterogeneity (Weyand & Kühn, 1990). Additional heterogeneity may result from the high susceptibility of arrestin to proteolysis at the N- and C-termini, deamination, and by contamination with  $\beta$ -arrestins or cone-specific arrestin(s).

*Drosophila* arrestin I and II are phosphorylated in  $Ca^{2+}$ -dependent processes (LeVine et al., 1990; Yamada et al., 1990), which, in vivo, may be regulated by light because  $Ca^{2+}$  levels increase upon illumination in invertebrates. Arrestin I and II exhibit distinct time courses of phosphorylation in vivo (Matsumoto & Yamada, 1991).

## Structure

Although the crystal structure of arrestin has not yet been elucidated, several biophysical methods give us a first glance of its structure. Furthermore, because arrestin does not have enzymatic activity, and its unique conformation is crucial for its interaction with photolyzed phosphorylated rhodopsin, these techniques are vital for routine examination of its integrity.

The purified protein exhibits a UV absorption band at 278–279 nm, which results from the absorbance of its 14 tyrosines and 1 tryptophan. Arrestin displays a fluorescence spectrum, which is dominated by tyrosine emission or shorter than expected emission from tryptophan. Observation of the tyrosine-like fluorescence is dependent on the purity and structural integrity of the protein. Denaturation of arrestin by guanidine hydrochloride or by temperature (a transition midpoint is 60 °C) results in a decrease in fluorescence at ~325 nm and an increase at ~340 nm, a useful marker of conformational transitions for this protein (Kotake et al., 1991). The native conformation of bovine retinal arrestin has been characterized by a variety of spectroscopic methods, by chemical modifications, and by secondary-structure prediction methods. From CD spectroscopy it was concluded that arrestin has predominantly  $\beta$ -conformation with an  $\alpha$ -helical region at the C-terminus (Shinohara et al., 1987a, 1987b). Expanded analysis of CD spectra down to 175 nm suggests that arrestin has virtually no  $\alpha$ -helical structure, ~40%  $\beta$ -structure, ~18%  $\beta$ -turns, and ~40% undefined structures (Palczewski et al., 1992a). Analysis by Fourier transform-infrared spectroscopy suggests that the secondary structure of arrestin comprises 56–63% of extended strands, 12–19% of turns and bends, 15% of  $\alpha$ -helices, and 10% of undefined and irregular segments (Garcia-Quintana et al., 1992).

## Ligands

We have found that heparin, dextran sulfate, phytic acid, and other highly phosphorylated inositols bind to arrestin and perturb its interaction with photolyzed phosphorylated rhodopsin (Palczewski et al., 1991c). The dissociation constants for arrestin and inositol phosphates are in the low micromolar range, and inositols are released when arrestin interacts with rhodopsin (Palczewski et al., 1991b). Despite recent reports, neither ATP, GTP, nor  $Ca^{2+}$  are ligands for arrestin; however, arrestin may bind  $Mg^{2+}$  (discussed in Palczewski & Hargrave, 1991). The reported ATP binding by arrestin, its intrinsic ATPase activity (Glitscher & Rüppel, 1991), and  $Ca^{2+}$ -binding properties (Hupertz et al., 1990) are probably the result of contamination of arrestin with rod outer segment membranes.

## Functions

Original biochemical observations by Kühn's group spurred great interest in arrestin function. Wilden et al. (1986) analyzed the mechanism by which arrestin deactivates  $G_T$ -mediated stim-

ulation of phosphodiesterase. Addition of purified arrestin to phosphorylated membranes, to which  $G_T$  and phosphodiesterase were reassociated, suppressed their phosphodiesterase-activating capacity; suppression could be as high as 98%, depending on the ratio between the amount of arrestin and the flash intensity. They proposed that arrestin binds to phosphorylated photolyzed rhodopsin and quenches its capacity to activate  $G_T$  and thereby phosphodiesterase.

Further biochemical evidence related to arrestin function soon followed. Bennett and Sitaramayya (1988) demonstrated that rhodopsin kinase alone terminates the activation of G protein and that arrestin speeds up the process at a relative concentration similar to that reported in the rod. In a reconstituted system composed of purified components, Benovic et al. (1987) found that when  $\beta_2$ -adrenergic receptor was stimulated by agonist, GTPase activity of G protein was only partially inhibited by the receptor phosphorylation, and the inactivation was augmented when excess of retinal arrestin was added.  $\beta$ -Arrestin displays striking specificity over arrestin with half-maximal inhibition occurring at a  $\beta$ -arrestin: $\beta_2$ -adrenergic receptor stoichiometry of about 1:1 (Lohse et al., 1992).  $\beta$ -Arrestin-2 is equipotent to  $\beta$ -arrestin-1 (Attramadal et al., 1992). This inhibitory property of  $\beta$ -arrestins seems to be related to the receptor phosphorylations by GRKs, but not by protein kinase A.

Inhibitors of arrestin/photolyzed phosphorylated rhodopsin interaction, specific antibodies, and overexpression of  $\beta$ -arrestin-1 were used to investigate arrestin functions in more complex or physiological systems. The mechanisms were studied in functionally intact, detached rod outer segments by testing the effect of heparin or phytic acid, inhibitors of arrestin binding to photolyzed phosphorylated rhodopsin, on light responses recorded in whole-cell voltage clamp (Palczewski et al., 1992b). Results obtained from these experiments suggest that photoisomerized rhodopsin is inactivated fully by multiple phosphorylation and that the binding of arrestin accelerates recovery by quenching partially phosphorylated rhodopsin. It is also conceivable that these inhibitors block the action of the splice variant of arrestin,  $p^{44}$ . Pippig et al. (1993) have generated transfected hamster ovary cell lines overexpressing the  $\beta$ -arrestin-1 and GRK3, and also expressing low or high levels of  $\beta_2$ -adrenergic receptors. For cells expressing high levels of  $\beta_2$ -adrenergic receptors,  $\beta$ -arrestin-1 and GRK3 become limiting for homologous receptor desensitization. For cells expressing low levels of the  $\beta_2$ -adrenergic receptor, receptor desensitization is accelerated. In another transduction system, odorants induce a transient elevation of cAMP, which activates a nonspecific cation channel and produces membrane depolarization. Preincubation of rat olfactory cilia, which have high levels of GRK3 and  $\beta$ -arrestin-2, with antibodies raised against GRK3 and  $\beta$ -arrestin-2 increased the odorant-induced elevation of cAMP and attenuated desensitization (Dawson et al., 1993). Electrophysiological analysis of mutations in *Drosophila* arrestin I and II demonstrate that they mediate rhodopsin inactivation and are essential for the termination of the phototransduction cascade. The depletion of arrestin by an excess of activated rhodopsin is responsible for a continuously activated state of photoreceptors (Dolph et al., 1993).

In addition to the function of arrestin in quenching G protein activation, evidence assessing the different, although possible complementary functions of arrestin is mounting. Further experimental approaches are needed for clarification of these ex-

periments. For example: (1) Wagner et al. (1988) have found that for rapid  $G_T$  deactivation in intact stacks of bovine rod outer segment disks, arrestin requires additional soluble proteins. (2) The insect arrestin homolog stimulates the light-induced phosphorylation of visual pigment by activation of a receptor protein kinase (Bentrop et al., 1993). (3) Zuckerman proposed that arrestin, in the presence of ATP, produced rapid turnoff by directly inhibiting activated phosphodiesterase (Zuckerman & Cheasty, 1986). Photoactivatable crosslinker was coupled to purified arrestin, and in the presence of ATP, the modified arrestin crosslinks to both phosphodiesterase and rhodopsin (Zuckerman & Cheasty, 1988). Furthermore, immobilized  $\gamma$ -subunit of phosphodiesterase binds not only the expected  $\alpha$ -subunit of the  $G_{T\alpha}$ -GTP complex,  $\alpha$ - and  $\beta$ -subunits of phosphodiesterase, but also arrestin (Qin & Baehr, 1993). (4) The addition of arrestin to rod outer segment membranes activates phospholipase C 2-3.4-fold (Ghalayini & Anderson, 1992). (5) A 48-kDa protein from the budding yeast *Saccharomyces cerevisiae*, which is structurally similar to retinal arrestin, associates with yeast DNA polymerase and with yeast DNA-replicative complex and stimulates polymerase activity. These findings suggest that the yeast arrestin-like protein may play a role in replication (Jeanson et al., 1991).

#### Structure of arrestin upon binding to the receptor

Schleicher et al. (1989) found that arrestin enhances the light-induced formation of the photoproduct metarhodopsin II (MII) from prephosphorylated rhodopsin. This effect is analogous to the known enhancement of MII (extra-MII) that results from selective interaction of MII with G protein. This interaction requires a multiple-point attachment to maintain rhodopsin in this conformation. Such a complex interaction involves several sites on arrestin and photolyzed phosphorylated rhodopsin (Ohguro et al., in prep.). Indeed, the cytosolic fragments of rhodopsin do not potently inhibit the interaction between phosphorylated activated receptor and arrestin (Krupnick et al., 1994), suggesting that multiple interactions between these 2 proteins must occur. Arrestin neither stabilizes nor interacts with MII when rhodopsin is unphosphorylated (Hofmann et al., 1992).<sup>2</sup>

The apparent dissociation constant for the MII-arrestin complex is  $\sim 50$  nM, and requires  $<200$  ms for binding to occur in physiological conditions. The temperature dependence of the MII-arrestin formation rate is very high, and the related Arrhenius activation energy is  $165 \text{ kJ mol}^{-1}$ . These results are consistent with a conformation change during the binding process (Schleicher et al., 1989). When bound to arrestin, heparin also mimics phosphorylated rhodopsin by similarly exposing arrestin to limited proteolysis, it suppresses the stabilization of MII, and restores arrestin-quenched phosphodiesterase activity (Palczewski et al., 1991c). However, arrestin interaction with heparin is much simpler than with photolyzed phosphorylated rhodopsin (Ohguro et al., in prep.).

The arrestin structure seems to be composed of 1 major structural domain extending from a few amino acids of the N-terminus

<sup>2</sup> Using gel filtration and a high arrestin/rhodopsin ratio, Gurevich and Benovic (1992; 1993) observed such binding; however, this may be an artifact due to partially phosphorylated rhodopsin present in most preparations from frozen retinas.

to ~360 residues. Both ends of the protein appear to be very accessible for digestion and thus are highly exposed (Palczewski et al., 1991a, 1991c). Arrestin lacking 30–40 C-terminal residues (generated by proteolysis, mutagenesis, or naturally occurring splice variant  $p^{44}$ ) binds specifically to photolyzed phosphorylated rhodopsin, to photolyzed rhodopsin, or to phosphorylated rhodopsin. This form of arrestin is also very tightly associated with membrane phospholipids, whereas arrestin containing the C-terminal region is readily soluble. This suggests that the C-terminal segment of arrestin plays an important regulatory role in maintaining arrestin in an inactive conformation, different than that of  $p^{44}$  (Palczewski et al., 1991a, 1994; Gurevich & Benovic, 1992). It was proposed that the N-terminal half of  $\beta$ -arrestin may play a key role in determining the receptor binding specificity (Gurevich et al., 1993).

Our understanding of precise changes in the arrestin conformation during binding to activated and phosphorylated receptor are highly speculative. Conceivably, arrestin is in an inactive conformation, where the negatively charged 40-amino acid long segment of the C-terminus forms an ion pair with a highly basic region around the midportion of the arrestin sequence. The C-terminus is replaced with the phosphorylated region of the receptor (that can be mimicked by heparin). If the receptor is activated, the stable complex is formed between cytosolic loops of the receptor and 2–12, 140–170, 230–240, and 270–300 regions of arrestin (Ohguro et al., in prep.). This order of events is necessary to provide the stability and selectivity of this multiattachment interaction between these 2 proteins.

## Conclusions

In conclusion, the current model of the potential role of arrestins in phototransduction is presented in Figure 2. Although this system has several specific steps, intellectually it is compatible with other transduction systems. Phototransduction begins by the photoisomerization of rhodopsin's chromophore, 11-*cis*-retinal to all-*trans*-retinal, resulting in conformational changes of rhodopsin ( $R \rightarrow R^*$ ). The active conformation ( $R^*$ ), identi-

fied with the MII photoproduct, initiates the excitation cascade of reactions by activating several thousand  $G_T$  molecules (reviewed by Hargrave et al., 1993). To terminate the light signal, MII is deactivated by 2 mechanisms: (1) rhodopsin kinase (RK) (Palczewski & Benovic, 1991) binds and phosphorylates MII at Ser-338, Ser-343, Thr-336, and Ser-334 ( $R^*P$ ) (McDowell et al., 1993; Ohguro et al., 1993b; Papac et al., 1993). The extent of phosphorylation was found to be limited by 2 mechanisms: (a) binding of arrestin to phosphorylated MII (1–3 phosphate groups per receptor); and (b) reduction of the photolyzed chromophore all-*trans*-retinal to all-*trans*-retinol prevents phosphorylation at more than 3 sites. Thus, no more than 1–3 phosphorylation sites appear to be physiologically significant (Ohguro et al., 1994). In contrast to the binding of arrestin and  $G_T$  to  $R^*$ , the binding of RK does not stabilize the MII photoproduct (Hofmann, 1993); however, RK competes with  $G_T$  for binding to photoactivated rhodopsin. Autophosphorylation of RK (RK-P) lowers the affinity of the kinase to MII (Buczylko et al., 1991; Pulvermüller et al., 1993). Because the binding of RK and arrestin partially overlap (Buczylko et al., 1991), the dissociation of the kinase is followed by the binding of arrestin (A). The binding of arrestin to photolyzed phosphorylated rhodopsin forms a complex that is resistant to dephosphorylation. The further decay reaction of MII results from the reduction of the photolyzed free all-*trans*-retinal into all-*trans*-retinol by disc membrane-associated retinol dehydrogenase (Hofmann et al., 1992). Once this reduction has occurred, arrestin dissociates from now inactivated P-opsin (O). The chromophore is regenerated and phosphates are removed by phosphatase 2A (PrP 2A) (Palczewski et al., 1989); (2) a rod outer segment-specific variant of arrestin  $p^{44}$  that is tightly associated with membranes (in contrast to arrestin, which is more cytosolic and present in other cells of the retina) binds to  $R^*$  (without phosphorylation) and prevents  $G_T$  activation. This splice variant of arrestin is a much more potent inactivator of phototransduction in vitro (Palczewski et al., 1994). Once the photolyzed chromophore is reduced,  $p^{44}$  dissociates and the receptor is regenerated with 11-*cis*-retinal (Palczewski et al., 1994). It remains to be estab-

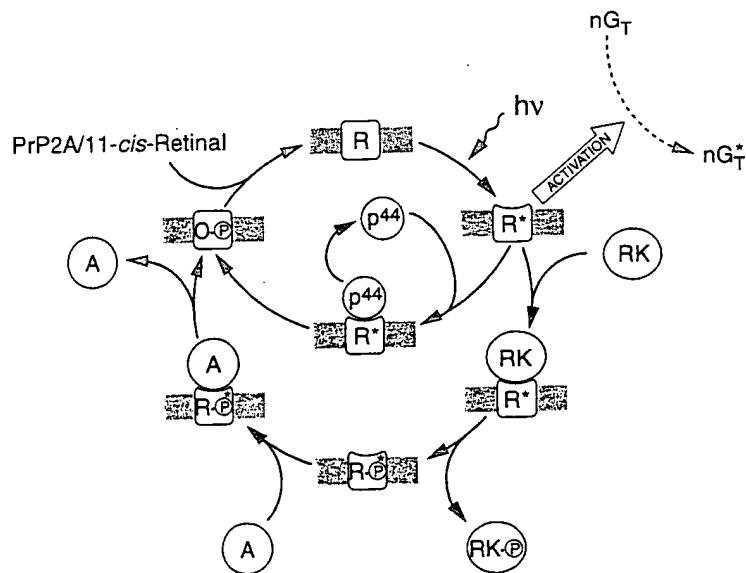


Fig. 2. Flux diagram of rhodopsin deactivation and regeneration with indicated functions of arrestin and its splice variant,  $p^{44}$  (see conclusions for details). Rhodopsin (R), when photoisomerized, assumes an active conformation, MII ( $R^*$ ).  $R^*$  activates hundreds (n) of  $G_T$  molecules and is quenched by 2 different pathways: (1)  $R^*$  is phosphorylated and binds arrestin (Arr); (2)  $R^*$  binds  $p^{44}$  (splice variant of arrestin), and the quenching is achieved without phosphorylation. However, we cannot exclude the possibility that  $R^*P$  is the only relevant form for arrestin and  $p^{44}$  binding in vivo. Both arrestin and  $p^{44}$  dissociate from the photoreceptor molecules when all-*trans*-retinal is reduced to all-*trans*-retinol. The regeneration cycle is accomplished when the phosphate is removed and opsin is regenerated with 11-*cis*-retinal. The model is a modification of the phototransduction cascade (Hofmann, 1993).

lished which of these 2 pathways is dominating at high and low levels of illumination.

### Note added in proof

Matsumoto et al. (1994) identified Ser 366 to be the phosphorylation site in *Drosophila* DarrII by Ca/calmodulin-dependent protein kinase, under in vivo conditions. This residue is conserved in DarrI but absent in vertebrate arrestins (Fig. 1).

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